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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/518,861	03/21/2005	Kenichiro Kosai	042-301	9980

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EXAMINER	
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ART UNIT	PAPER NUMBER
1633	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/518,861	KOSAI ET AL.	
	Examiner	Art Unit	
	MARIA LEAVITT	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *27 November 2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-4,6,7,14-18,21,24,27,30,33,34 and 36* is/are pending in the application.

4a) Of the above claim(s) *21,24 and 27* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-4,6,7,14-18,30, 33, 34 and 36* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *04-22-2008*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Detailed Action

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 11-27-2008 has been entered.
2. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.
3. Claims 1-4, 6, 7, 14-18, 21, 24, 27, 30, 33, 34 and 36 are pending. Claims 5, 8-13,19-20, 22-23, 25-26, 28-29, 31-32, 35 and 37-38 have been cancelled, and claims 1, 6, 7, 18, 21, 24, 27, and 30 have been amended by Applicant's amendment filed on 11-27-2008.
4. Claims 21, 24, and 27 directly or indirectly depend from claim 11, now cancelled. Therefore, claims 21, 24 and 27 have not been further treated on the merits
5. Accordingly, claims 1-4, 6, 7, 14-18, 30 33-34, and 36 are currently under examination to which the following grounds of rejection are applicable.

Response to arguments

Rejections maintained in response to Applicants' arguments or amendments

Claim Rejections - 35 USC § 103

Claims 1-4, 6, 7, 14-18, 30, 33, 34 and 36 remain rejected under 35 USC 103 as being unpatentable Vallier et al, (PNAS, 2001, 98:2467-2472) in view Ong et al., US Patent No:

6,777,235, Date or Patent Aug. 17, 2004) and Rybkin et al., (Biol. Chem., 15927-15934, 2003) and further in view of Yamamote et al., (Oncogene 2002, 899-908).

Vallier et., (PNAS, 2001, 98:2467-2472) teaches a system for conditional gene expression in undifferentiated embryonic stem cells and their derivatives *in vitro*, said method relies on tamoxifen-dependent Cre recombinase-loxP site-mediated recombination and bicistronic gene-trap expression vectors that allows transgene expression from endogenous cellular promoters (Abstract). Specifically, Vallier discloses a method of gene trap comprising transfection with two recombinant vectors, a first and a second recombinant vector. The first recombinant vector, i.e., a first trap vector (e.g., *pGTEV-Cre-ER*) comprising a SA, a βgeo as a reporter gene, an IRES, **Cre-**RE and a stop signal (Fig. 1A), is stably integrated down stream an endogenous promoter. Thus after integration downstream of an endogenous promoter, βgeo and Cre-ER are co expressed from a single transcript. In addition the recombinase (e.g., CRE) is activated by 4'OH-tamoxifen (4'OHT) and not endogenous estrogens. The second recombinant vector, i.e., the reporter vector (e.g., *pCAG-lox-STOP-EGFP*), comprises a unit of the following genes: **a constitutively activated promoter** (e.g., CAG) (**Current claim 4**), **a first loxP sequence**, a drug resistance gene (hygro), a stop sequence, **a second loxP** and a reporter gene, the expression of which is prevented by tandemly repeated stop-of-transcription sequences flanked by loxP sites. (p. 248, col.1, paragraphs 2-4; p. 2469, col. 1, Fig B; p. 2472, col. 1 paragraph 2). This system allows for 4'OHT –dependent expression of EGFP after transient transfection of ES cells with the pPGK-lox-STO-lox-EGFP. Thus, expression of the recombinase in the pGTEV-Cre-ER of Vallier, which is constitutively expressed under the control of an endogenous promoter, is 4'OHT dependent whereas expression of the recombinase in the instant

invention is under the control of a promoter specifically expressed in the target cell differentiated from an embryonic stem cell (e.g., Nkx2.5). The *pCAG-lox-STOP-EGFP* of Vallier and the first recombinant vector of the invention comprising sequentially from a 5' end, a gene (e.g., neo, stop) having recombinase recognition sequences on both ends (e.g., loxp sites), and a fluorescence protein gene (EGFP) are essentially the same. Quantification of EGFP- expressing undifferentiated embryonic stem cells and their derivatives was performed by FACS (p.2468, col. 1, paragraph 1). (**Current claims 1-3, 6, 14-16, 18 and 33, in part**). Further, Vallier et., teaches a kit including the first DNA construct and, a second DNA construct (col. 6, lines 30-34) (Current claims 30) .

Vallier et.al, does not specifically teach cell-specific dependent Cre recombinase-activty.

However, at the time the invention was made, Ong et al., is an exemplified prior art that teaches that it is routine or well –established in the art to use cell-type or tissue type restricted expression of certain promoters operatively associated with a recombinase gene in combination with a gene of interest that has been flanked by recombinase recognition sequences, wherein the recombinase is expressed under the control of the cell-type or tissue type specific promoter and when expressed results in the excision of the gene of interest. (See for example col. 10, lines 40-45). Moreover, in column 8, lines 10-40, Ong et al., discloses a list of tissue or cell specific promoters. Ong et al., teaches that invention may be used to identify tissue or cell type specific genes and cells are then screened for activity of the indicator which will occur in the cell or tissue type in which the promoter is functional in any animal from which embryonic stem (ES) cell lines may be obtained (col. 6, lines 48-62) Further, Ong et., teaches a kit including the first DNA construct and, a second DNA construct (col. 6, lines 30-34) (**Current claims 30, and 1-3,**

6, 14-16, 18, 33 36, in part). Additionally, Rybkin et al., (JBC 2003, 15927-15934) teach inducible swich from proliferation to differenciation of mouse ventricular myocardium by conditional expression of simian virus 40 large T-antigen (Tag) under the control of the early cardiac promoter Nkx2.5 and Cre-mediated recombination (p. 15927, col. 1, paragraph 1, p. 15928, col. 1, paragraph 4) (**Current claims 7, 17 and 34**).

The combined teachings of Vallier, Ong and Rybkin fail to disclose gene transfer with adenovirus vectors.

Comment [a1]: but does Behfar et al. actually teach the Nkx 2.5 promoter sequence and also teach the use of the promoter sequence to express heterologous genes???

However, at the time the invention was made, Yamamote et al., successfully demonstrate conditional expression of the HST-1/FGF-4 gene in the testis of mice using the Cre/lox system by administration of a recombinant adenovirus expressing the Cre recombinase *in vivo* (p. 900, col. 1, paragraph 3). Moreover, Yamamote et al., teaches that when a sufficient amout of a Cre si supplied provided by the adenovirus-carrying the Cre gene, the stuffer sequence is excised and the the HST-1/FGF-4 gene under the control of a CAG promoter is expressed

Therefore, it would have been *prima facie* obvious for one of ordinary skill in the art at the time the invention was made in an attempt to take advantage of the cell-type or tissue-type restricted expression of certain promoters operatively associated with a recombinase gene in combination with a gene of interest as disclosed by Ong and Rybkin to use any of the specific cell-type or tissue- type promoters known in the art , as exemplified by the cited references, in the bicistronic trap vector taught by Vallier to replace the tamoxifen-dependent Cre inducible recombinase with an alternate cell specific promoter-dependent Cre activity to visualized cells differentiated from murine ES cells, particularly because Ong et al., teaches the advantage of using a cell-type or tissue type restricted promoter operably linked to a marker gene to screening

cell or tissue specific genes in any animal from which ES cell lines may be obtained. Further, one of ordinary skill in the art would have been motivated to use a recombinant adenovirus vector rather than plasmids to stably express a gene transferred into a host cell, e.g, Cre gene, particularly because Yamamote et al., successfully teachess expression of the gene of interest when Cre is supplied provided by a recombinat adenovirus-vector carrying the Cre gene. The manipulation of previously identified DNA fragments and cell transformation systems is within the ordinary level of skill in the art of molecular biology. Moreover, based on the detailed teachings of the Vallier, Yamamote, Rybkin and Ong and the high level of skill in the art of molecular cloning, the skilled artisan would have had a reasonable expectation of success in generating a method for selectively isolating or visualizing a target cell using adenoviruses carrying vectors as claimed.

Reply to applicant arguments as they relate to rejection of Claims 1-4, 6, 7, 14-18, 30, 33, 34 and 36 under 35 USC § 103.

At pages 6 and 7 of the Remarks filed on 11-27-2008, in relation to the Vallier disclosure, Applicants essentially argue that in the Vallier reference, a target cell differentiated from an ES cell is not identified and purified by using EGFP as a marker. In addition Applicants argue that the Cre-loxP system in the attached reference of Indra et al., is different from that in the present invention. Insofar as the Rybkin and Ong references, Applicants essentially allege that claim 1 has been amended to encompass only a DNA of interest not integrated into the target cells, in contrast to the teachings of Rybkin and Ong.

In response to Applicants' arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). In contrast to applicants' argument, Vallier clearly discloses that ES cells express EGFP after transfection with *pCAG-lox-STOP-EGFP* (Table 1) and that quantification of EGFP- expressing undifferentiated embryonic stem cells and their derivatives was performed by FACS (p.2468, col. 1, paragraph 1). In addition, the system was useful to visualize and isolate by FACS ES cells that are induced to differentiate either by incubation with 10^{-6} M retinoic acid for 2 days or culture with leukemia inhibitory factor-deprived culture medium (p. 2468, col. 1, paragraph 5; See Watson et al., submitted by Applicants at page 257, col. 2). Note that the preamble of claim 1 does not add any active method steps. Hence, the preamble is not limiting when the claim body describes a structurally complete invention such that deletion of the preamble phrase does not affect the structure or steps of the claimed invention. That is case here where the active method steps of the invention do not place any limitation on isolating or visualizing cells. Insofar as the disclosure of Ong and Rybkin teaching that the DNA of interest is integrated into target cells, this is not dispute. However, Rybkin additionally teaches introduction of Cre recombinase into cardiac cells by adenoviral delivery (See Abstract). Likewise, Yamamote et al., evidences transfection with recombinant adenoviruses expressing the Cre recombinase. So if integration of a gene of interest by a plasmid (e.g., as disclosed by Vallier) leads to stable expression of the incorporated gene, delivery of a gene of interest by an adenoviral vector should be reasonably expected to stably express the gene of interest. It is noted that the instant invention does not place any limitation in

relation to expression of any of the first and second recombinant DNA by an adenovirus that is in episomal form. Furthermore, as stated at page 5 of previous office action of 02-22-2008, the mere change of a plasmid (e.g., leads to integration of the gene of interest into the host cell) by an adenovirus vector (e.g., remains in episomal form) in the transfection of a target cell to stably express of a gene of interest has no patentable significance unless a new and unexpected result is produced.

New Grounds of Objection/ Rejection

Claim Objections

Claim 4 is objected to because of the following informalities: abbreviations such as CA should be spelled out at the first encounter in the claims. Appropriate correction is required

Claims 15-17 are objected to because of the following informalities. Each of these claims refers to the product (embryonic stem cell) without referring to a preceding by use of a definite article "The". The use of a definite article in this context is grammatically incorrect, i.e. the indefinite article –an or a-- should be used in this context. For example, " The" (claim 15 or 16) "embryonic stem cell … DNA as defined in claim 1…" should be --An-- "embryonic stem cell …". Appropriate correction is required.

Claim Rejections - 35 USC § 112- Second Paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter, which the applicant regards as his invention.

Claims 1-4, 6, 7, 14-18, 30, 33, 34 and 36 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 recites the limitation "strongly expressed by said first promoter". The phrase "strongly expressed" is a relative concept that requires a comparative reference as to define increase in the maker expression. However, it is unclear how much the expression of the fluorescence protein is increased and relative to what. As such, the metes and bounds of the claims cannot be determined.

Additionally, claim 1 is indefinite in the reciting "in this order" in lines 6 and 9. There is no antecedent basis for a corresponding order in the claim. Therefore, the metes and bounds of "in this order" are indefinite.

Claim is vague and indefinite in its recitation of "a fluorescence protein of a target cell differentiated from an embryonic stem cell". It is unclear whether the fluorescence protein is selectively expressed in the cell differentiated from the embryonic stem cell or it is a marker encoded by the first recombinant DNA operationally linked to a promoter to distinctively drive a fluorescence protein expression in the differentiated cell.

Furthermore, claim 1 is vague and indefinite in the reciting "with an adenovirus vector as an episomal form" in lines 9-10. It is unclear whether the first recombinant DNA and second recombinant DNA are separately transferred into the differentiated embryonic stem cells or they are each comprised in a gene encoded by an adenovirus vector, or alternatively, only one of the recombinant DNAs is encoded by the adenovirus vector and the other is encoded by a different gene vector. Thus the meaning and the metes and bounds of the claim as whole are unclear.

Moreover, claim 1 is incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. While all of the technical details of a method need not to be recited, the claims should include enough information to clearly and accurately describe the invention and how it is to be practice. Claim 1 is drawn to a method for isolating or visualizing a target cell *in vitro* comprising transferring a first recombinant DNA and a second recombinant DNA with an adenovirus vector as an episomal form. The steps listed in the method do not result in the selective isolation or visualization of a target cell. Thus it is not apparent as to under what structural or functional parameters the transferring of a first and a second recombinant DNA is indicative or correlative to the preamble of the claims.

Claims 2-4, 6, 7, 14-18, 30, 33-34 and 36 are indefinite insofar as they depend from claim 1.

Other art for comment

The following art are cited on a PTO-892 to complete the record:

2) Indra et al., *Nucleic Acids Res*. 1999, pp. 4324-7.

Conclusion

Claims 1-4, 6, 7, 14-18, 30, 33-34 and 36 are rejected.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Maria Leavitt whose telephone number is 571-272-1085. The examiner can normally be reached on M-F.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach, Ph.D can be reached on (571) 272-0739. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1633; Central Fax No. (571) 273-8300. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

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/Maria Leavitt/

Maria Leavitt, PhD
Examiner, Art Unit 1633